Amendment Dated March 8, 2007

Reply to Office Action of December 8, 2006

<u>Amendments to the Claims:</u> This listing of claims will replace all prior versions, and listings, of claims in the application

Listing of Claims:

1. (Currently Amended) A method of determining a binding capacity of a surface, the method comprising:

providing the surface, said surface comprising containing a first reactive moiety;

contacting the surface with providing a fluorophore comprising a fluorescent moiety and a second reactive moiety adapted to emit a detectable signal, thereby causing a reaction between the first and second reactive moieties and forming a linking bond or group that binds the fluorescent moiety to the surface;

reacting the fluorophore with the reactive moiety to form a linking bond between the fluorophore and the reactive moiety;

cleaving a cleavable the linking bond or group, thereby to liberat[e]ing the fluorescent moiety from the surface;

exposing the liberated fluorescent moiety to exciting radiation; and

measuring detecting the detectablea signal emitted by the liberated fluorescent moiety; and

<u>calculating</u>to determine the binding capacity of the surface <u>from the strength of the signal</u>.

2. (Currently Amended) A method of determining a binding capacity of a surface, the method comprising:

providing the surface containing a reactive moiety;

providing a fluorophore comprising a fluorescent moiety adapted to emit a detectable signal;

reacting the fluorophore with the reactive moiety to form a linking bond between the fluorophore and the reactive moietyThe method of claim 1, wherein the linking bond or group the cleavable bond and is comprises a disulfide bond or an aromatic azo group bond and wherein the step of cleaving the linking bond or group comprises cleaving the disulfide or aromatic azo bond;

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cleaving a cleavable bond to liberate the fluorescent moiety; and detecting the detectable signal to determine the binding capacity of the surface.

- 3. (Currently Amended) The method of claim 2, wherein the <u>linkingeleavable</u> bond <u>or group comprises</u> a disulfide bond.
- 4. (Currently Amended) The method of claim 2, wherein the <u>linking bond or group</u> <u>comprises an aromatic azo group is</u>-represented by <u>athe</u> formula:

$$-R^2-N=N-$$

wherein R<sup>2</sup> is an <u>divalent</u> aromatic <del>compound</del> moiety selected from the group consisting of a heterocyclic groups and <del>an</del>-electron-deficient aromatic groups.

5. (Currently Amended) The method of claim 2, wherein the fluorophore is a thiol-containing fluorescent structurecompound represented by athe formula:

wherein Fl iscomprises the fluorescent moiety, and wherein Fl-SH is a member selected from the group consisting of fluorescent L-cysteine derivatives bearing fluorescent substituents, BODIPY-L-cysteine, and compounds wherein Fl comprises a fluorescein moiety and derivatives thereof.

6. (Currently Amended) The method of claim 5, wherein the thiol-containing fluorescent structurecompound is a member selected from the group consisting of:

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7. (Currently Amended) The method of claim 5, wherein the thiol-containing fluorescent structurecompound is

8. (Currently Amended) The method of claim 5, wherein the thiol-containing fluorescent structurecompound is:

9. (Currently Amended) The method of claim 2, wherein the fluorophore is a thiol-reactive fluorescent structurecompound represented by athe formula:

wherein X is a member-selected from the group consisting of CI,  $SO_3(C_1-C_6 \text{ alkyI})$ , and  $S-R^2$ , wherein  $R^2$  is a heterocyclic group or an electron-deficient aromatic group.

10. (Currently Amended) The method of claim 9, wherein  $R^2$  is a pyridyl group or a phenyl group substituted with one or more electron-withdrawing substituents.

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11. (Currently Amended) The method of claim 9, wherein the thiol-reactive fluorescent structurecompound is a member-selected from the group consisting of:

$$(H_{3}C)_{2}N$$

$$(H_{3}C)_{2}N$$

$$C000^{9}$$

$$NH. and$$

$$NMc_{2}$$

$$O$$

- 12. (Currently Amended) The method of claim 2, wherein the fluorophore further comprises a functional group, wherein the functional groupsecond reactive moiety is bound to the fluorescent moiety by the cleavable bond and is reacted with the reactive moiety to form an uncleavable bond such that cleaving predominantly occurs at the cleavable bond disulfide bond or aromatic azo bond.
- 13. (Currently Amended) The method of claim 12, wherein the <u>second reactive</u> moietyfunctional group is a member-selected from the group consisting of an amino group, a thiol group, a protected thiol group, and an epoxy group.
- 14. (Currently Amended) The method of claim 2, wherein the surface is a member selected from the group consisting of a polymer, a metal, a biomaterial, a ceramic, and a semiconductor.
- 15. (Currently Amended) The method of claim 14, wherein the <u>surfacepolymer</u> is polyurethane.
- 16. (Currently Amended) The method of claim 2, wherein the <u>first</u> reactive moiety is a thiol, a thiol-reactive group or a group adapted to be converted into a thiol or a thiol-reactive group.

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17. (Currently Amended) The method of claim 2, wherein the <u>first</u> reactive moiety is a thiol group or an amino group.

- 18. (Currently Amended) The method of claim 172, wherein the <u>first</u> reactive moiety is <u>a</u> reaction product of a surface thiol group or surface amino group further reacted with 5,5'-dithio-bis(2-nitrobenzoic acid) or succinimidyl 3-(2-pyridyldithio) propionate.
- 19. (Currently Amended) The method of claim 2, wherein the <u>first</u> reactive moiety is a dithio group.
- 20. (Currently Amended) The method of claim 2, wherein the <u>disulfide bond or aromatic azocleavable</u> bond is cleaved by using a reducing agent selected from the group consisting of dithiothreitol,  $\beta$ -mercaptoethanol, mercaptoethylamine hydrochloride, a borohydride, and a phosphine.
  - 21. (Original) The method of claim 20, wherein the borohydride is sodium borohydride.
- 22. (Currently Amended) The method of claim 20, wherein the phosphine is a member selected from the group consisting of tris(2-cyanoethyl)phosphine, tris(2-carboxyethyl)phosphine and trimethylphosphine.
- 23. (Withdrawn) A kit for practicing of method of claim 2, the kit comprising a fluorophore.
- 24. (Withdrawn) The kit of claim 23, wherein the fluorophore comprises the fluorescent moiety and a linking bond precursor.
- 25. (Withdrawn) The kit of claim 23, wherein the linking bond precursor is adapted to form a cleavable disulfide bond or an aromatic azo group.
  - 26. (Withdrawn) The kit of claim 25, wherein the linking bond precursor is —SH.
- 27. (Withdrawn) The kit of claim 25, wherein the linking bond precursor is represented by a formula:

-s-x

wherein X is a member selected from the group consisting of Cl,  $SO_3(C_1-C_6 \text{ alkyl})$ , and  $S-R^2$ , wherein  $R^2$  is a heterocyclic group or an electron-deficient aromatic group . . .

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28. (Withdrawn) The kit of claim 23, wherein the fluorophore further comprises a functional group, wherein the functional group is bound to the fluorescent moiety by the cleavable bond and is adapted to react with the reactive moiety to form an uncleavable bond.

- 29. (Withdrawn) The kit of claim 28, wherein the functional group is a member selected from the group consisting of an amino group, a thiol group, a protected thiol group, and an epoxy group.
  - 30. (Withdrawn) The kit of claim 28, wherein the uncleavable bond is an amide bond.